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DEVELOPMENT OF PARATYPHOID-ENTERITIDIS GROUP IN VARIOUS FOODSTUFFS

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In the study of so-called "food poisoning," more properly termed food infection, by members of the paratyphoid-enteritidis group, it was deemed advisable to gain some idea of the ability of the various members of this group to multiply in miscellaneous food products. Numerous instances are on record in which milk and certain meat products, such as meat pies, the English brawn, meat soups and gelatin have served as excellent culture mediums for these forms, with resultant "food poisoning" outbreaks. The summary given by Savage [1] of the outbreaks of "food poisoning" in England shows that while meat products comprise the class of food most commonly incriminated, others have occasionally been held responsible. In the present work various vegetable foods and fruits as well as several meat products were used. In each case the aim has been to determine, at several different temperatures, the rapidity of development and the ability to spread from the point of inoculation throughout the foodstuff.

Another phase of this subject which seems important from the standpoint of prevention is that of the physical condition of the infected food. May foods in which members of the Gaertner group have had an opportunity to multiply exhibit physical evidence of spoilage to such an extent that it would be forced on the consumer's attention and cause him to reject the food as unfit for consumption? Since many of the past reports of outbreaks following food infection have failed to record information bearing on this point, it has been given some attention in the present investigation.

The question of the possible production by these organisms of soluble toxin in various foodstuffs is of paramount importance, but it could not be included in the present investigation, as no strains were available which consistently caused illness when fed to experimental animals.

As a rule, canned foods, particularly vegetables and fruits, were used as the source of test materials, since the fresh products were not

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[1] Food Poisoning and Food Infections, 1920, p. 76.

available at all times. The hydrogen-ion concentration of such canned products is greater than that of the corresponding unheated foods,[2] and it is possible that in some cases at least the use of fresh materials might have given different results. The procedure here followed, however, would simulate closely conditions in the household where after the partial consumption of cooked food the remainder is set aside for future use. The foods to be inoculated were contained either in flasks or in large glass dishes, according to the nature of the product. When working with liquid or semiliquid foods a measured quantity, usually 50 or 100 c c, was introduced into 300 c c Erlenmeyer flasks and sterilized in the autoclave for 30 minutes at 15 pounds pressure. In some instances certain modifications of this procedure were necessary, for example, in the case of evaporated milk and the various fruits which are packed in sugar syrups. These were removed aseptically from the original can in order to avoid sterilization. Solid substances, such as meat, were held in large glass dishes. Aerobic conditions were maintained throughout the work.

Inoculation was made from a 24-hour broth culture diluted to such an extent that on the introduction of one loopful the test substance should contain only a few thousand organisms per c c. When testing the ability of the paratyphoid group to develop and spread in solid materials, such as cooked meat, inoculation was made by placing one loopful of a diluted broth culture at one spot on the surface and then, with a straight wire, stabbing through this well into the material. By this procedure the greater number of organisms in the inoculum were left on the surface, while a few were carried into the interior of the mass. In every case tubes containing a known amount of sterile water were also inoculated and an estimate of the numbers of cells in the inoculum was obtained by plating.

Specimens were held at several different temperatures, 37 C., 20 C., and in an icebox the temperature of which varied from 6 to 9 C. At regular intervals observations were made on the physical condition of the food, and samples were withdrawn for plating and for determination of the hydrogen-ion concentration. The hydrogen-ion concentration was determined only roughly by mixing in the depressions of a porcelain test plate 0.5 c c of the food product with one drop of a suitable indicator. Dextrose litmus agar was used for plating. Wherever possible the plate counts are expressed as organisms per c c of the liquid part of the food product. The solid foods were examined by removing small pieces of the material—about 1 gm.—weighing and grinding with sterile sand in a mortar. This was followed by dilution and plating. The location and distance from the site of inoculation of the removed pieces was always recorded.

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[2] Bigelow, W. D., and Cathcart, P. H.: National Canners Assn. Bull. 17-L, 1921.

On examination of the cooked meats, which had not been sterilized previous to inoculation, it was found that the paratyphoid colonies were sufficiently distinct in appearance on dextrose litmus-agar plates so that no confusion resulted in differentiating them from the miscellaneous organisms surviving the cookng of the meat. As an additional precaution colonies were fished from time to time, transferred to Russell's medium, and agglutination tests were made to check the identity of the cultures thus obtained.

TABLE 1

DEVELOPMENT OF B. ENTERITIDIS (BRIGHTON) IN SOME MISCELLANEOUS FOODSTUFFS

Temperature of Incubation	No. of Samples	Original Inoculum	Plate Counts After				P_H	
			24 Hours	48 Hours	4 Days	7 Days	Before Inoculation	At 7th Day
Sauerkraut:								
237 C.	1	1200*	Sterile	Sterile	Sterile	....	3.4	
20° C.	1	1200	Sterile	Sterile	Sterile		to	
6-9° C.	1	1200	....	Sterile	Sterile		3.6	
Tomatoes:								
37° C.	2	2650	27,000,000*	49,000,000	26,000	Sterile	4.0	4.0
20° C.	2	2650	90,000	800,000	18,000,000	19,800,000	to	to
6-9° C.	2	2650	3,800	7,000	4,500	1,500	4.4	4.4
Spinach:†								
37° C.	2	2400	144,000,000	3,900,000	Sterile	Sterile	About	5.2
20° C.	2	2400	19 000	3,200,000	52,000,000	1,300,000	5.2	to
6-9° C.	2	2400	2,600	2,100	2,200	4,200		5.4
String beans:†								
37° C.	2	1900	40,000.000	14,000,000	10,000	Sterile	5.2	5.0
20° C.	2	1900	520,000	195,000,000	165,000,000	155,000,000	to	5.0
6-9° C.	2	1900	....	1,460	1,500	630	5.4	5.3
Corn:								
37° C.	2	2300	216,000,000	2,460,000	Sterile	Sterile	5.8	5.0
20° C.	1	2300	66,000,000	380,000.000	230,000,000	158,000,000	to	5.0
6-9° C.	2	2300	9,600	11,000	....	95,000	6.2	6.0
Peas:								
37° C.	2	2800	3,200,000.000	5,900,000,000	2,960,000,000	350,000,000	5.8	8.6
20° C.	2	2800	4,000 000	1,200,000.000	2,800,000,000	4,000,000,000	to	7.6
6-9° C.	2	2800	7,000	....	37,000	110,000	6.0	6.0
Evaporated milk:								
37° C.	2	7000	1,500,000,000	3 400 000,000	1,200,000,000	630,000,000	5.2	7.2
20° C.	2	7000	340,000	230,000,000	4,200,000,000	3,700,000,000	to	6.6
6-9° C.	2	7000	5,600	....	6,800	25,000	5.4	5.4

* Counts are expressed as numbers per c c of the liquid portion of the food product.

† Several additional samples of spinach and string beans have given discordant results, showing a gradual decline in numbers instead of a multiplication. These samples had a slightly greater hydrogen-ion concentration than those shown in the above table.

The types of the Gaertner group regularly employed throughout this work were a strain of B. enteritidis isolated by Savage and Forbes[3] from a human food poisoning outbreak at Brghton, England, and strain "rabbit 1371," a representative of the animal paratyphosus B group, obtained by Litch and Meyer[4] during the course of an outbreak among rabbits. Other organisms employed from time to time included the Jordan 210[5] and Rowland strains of human B. paratyphosus B, or B. schottmülleri, and the Calf-typhus 1 strain described by Ten Broeck.[6] The behavior of these various cultures was, as a rule, quite similar, so that the results with one only are presented in detail.

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[3] Jour. Hyg., 1918, 17, p. 460.

Table 1 shows the development of B. enteritidis in various vegetable foods and in evaporated milk. With the exception of the highly acid sauerkraut, this organism is able to multiply, at least to a certain extent, in all of the products tested. The growth in tomatoes was rather surprising in view of the acidity of the product. One of the noteworthy features of the table is the fact that at 37 C. there occurred in many of the products a rapid growth during the first 24 or 48 hours, followed by an abrupt decline in numbers. This was true of tomatoes, spinach, corn, and string beans. In some instances no viable organisms could be found in 1 c c quantities at the fourth day after inoculation. This rapid decrease in numbers following an initial multiplication is correlated with a high hydrogen-ion concentration and occurred in those foods in which the acidity is increased by the metabolic activities of the organism and also in those which maintain during growth an orginally high hydrogen-ion concentration. In peas and evaporated milk the change in the hydrogen-ion concentration was toward an alkaline reaction, in contrast to the foodstuffs mentioned. Also, in these 2 products the decline in the number of organisms, after the height of the growth curve was reached, is gradual and not abrupt. Despite the increased percentage of solids in the evaporated milk, the growth of the Gaertner group and the resultant changes in the hydrogen-ion concentration were quite similar to those taking place in the ordinary skim milk medium. Other types of the Gaertner group gave similar results, although the decrease in numbers in the acid foodstuffs was in most cases less abrupt than that exhibited by the Brighton strain of B. enteritidis. For example, viable organisms of the "rabbit 1371" strain were frequently present in small numbers after 7 days at 37 C. At 20 C. the rate of growth of all the cultures tested was slower, and the period of maximum development was not followed by the speedy destruction which took place at the higher temperature. In the icebox there was a slow multiplication in several of the foodstuffs.

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[4] Jour. Infect. Dis., 1921, 28, p. 27.

[5] Ibid., 1917, 20, p. 457.

[6] Jour. Exper. Med., 1920, 32, p. 19.

In a number of instances other organisms, such as the colon bacillus, were used together with the paratyphoid strains to inoculate the foods. In such cases B. coli grew more rapidly than did the paratyphoids, although the presence of the latter could usually be detected by streaking Endo medium with some of the material in question. The growth of the colon bacillus was accompanied frequently by the production of gas and sour odors in many of the foods, whereas the paratyphoids alone usually gave much less evidence of their presence.

In addition to the food products already enumerated, several fruits were used. Samples of pineapples, respberries, cherries and pears were inoculated with approximately the same number of organisms as were the vegetables. In every case there was a rapid decline in numbers, and 1 c c quantities of the product frequently yielded negative results after 24 hours. This reduction was most rapid at 37 C. and least so at 6 to 9 C. At the latter temperature small numbers of viable cells would sometimes be detected after 48 hours or more. The several strains differed slightly in their resistance to the destructive effect of the organic acids of the fruits, although this difference was not marked. The hydrogen-ion concentrations of the fruits were: pineapples, P_H 3.4-3.6; pears, P_H 4; cherries P_H 3.8-4; that of the raspberries could not be determined with indicators due to the color of the product. The figures given by Bigelow and Cathcart,[2] as determined electrometrically, vary from P_H 3.23-3.69.

When determining the ability of B. enteritidis to develop in meat products, corned beef, cooked ground meat (Hamburger steak), and canned salmon were used. The detailed results secured with samples held at several temperatures are presented in table 2. Here it is seen that in all of these products, under favorable temperature conditions, there is a multiplication at the point of inoculation and more or less spreading over the surface and throughout the interior. The moisture content and texture of a food quite naturally determine the extent to which the organisms may spread. Thus in a compact, comparatively dry product, such as corned beef, the area throughout which B. enteritidis was found during the first period of development was considerably smaller than in the softer foodstuffs containing liquid. A comparison of the results secured at the different temperatures is of interest in connection with the potential danger of contamination of a large mass of food from one original point. Thus, in Hamburger steak, within a period of 12 hours at 37 C., there is a rapid multiplication at the point of inoculation while penetration to other parts of the meat has not occurred. In contrast to this, after 24 hours great numbers of organisms are to be found at some distance from the original point of inoculation. At 20 C., the period of maximum growth at the point of inoculation occurs after the first 12 hours, while even after 48 hours the organisms have failed to spread to any extent throughout the meat. It is evident that, while multiplication may take place at the point of inoculation under unfavorable temperature conditions, it is only at the optimum temperature that the rapid penetration and spreading of the organisms throughout the meat occurs. Obviously, then, accidentally contaminated meat held at body temperature would constitute a much greater source of danger than if held at a lower temperature, due not merely to the increase in numbers at the point of contamination but especially to the spreading of the organisms throughout the product.

TABLE 2

DEVELOPMENT OF B. ENTERITIDIS (BRIGHTON) IN SEVERAL TYPES OF MEAT PRODUCTS

Sample Held at	Original Inoculum	Interval After Inoculation	Location and Approximate Distance from the Point of Inoculation					
			Point of Inoculation *	Surface			Interior	
				1 Cm.	3 Cm.	5 Cm.	1 Cm.	3 Cm.
Corned Beef:		Hrs.						
37° C.	4,800	24	3,600,000	270,000	0	0	6,000	0
		48	56,000,000	Contaminated	2,700,000	81,000	10,000,000	0
20° C.	4,800	24	5,800	80	0	....	40	0
		48	18,000	0	0	....	....	0
Hamburg Steak:								
37° C.	10,000	12	570,000,000	37,000.000	0	0	21,600,000	210
		24	26,400,000,000	11,400,000,000	230,000,000	180,000,000	17,400,000,000	300,000,000
		48	....	....	3,800,000,000	3,000,000,000	....	1,700,000,000
20° C.	10,000	12	12,200	330	0	....	20	
		24	200,000,000	22,400	0	510	11,000,000	0
		48	4,600,000,000	7,400,000	0	....	24,000,000	21,000
Red Salmon:								
37° C.	6,600	12	780,000,000	9.100,000	0	0	60,000,000	3,700
		24	3,500,000,000	3,100,000,000	860,000,000	48,000,000	355,000,000	Contaminated
20° C.	6,600	24	3,600.000	0	0	0	9,300	0
		48	7,700,000	6.600	2,500	0	230,000	40,000
9° C.	6,600	24	1.600	0	0	0	200	0
		48	2,500	0	0	0	600	0
		96	5.900	20	0	0	10	0

* Counts are expressed as number of B. enteritidis per gram.

Throughout this work it was observed regularly that in most of the foods the paratyphoid-enteritidis group failed to give any visible evidence of growth. Peas were the most noteworthy exception, for the organisms when growing in this product formed a thin white surface pellicle. In several of the food products, especially peas and corn, there was some evidence of gas formation. This might readily escape the notice of the consumer, however, unless the gas were prevented from escaping. With the exception of these few cases the contaminated foods regularly appeared normal. Also, no abnormal odors could be detected in any instance. From these observations it may be stated that extensive multiplication of the Gaertner group may take place in most foodstuffs with little or no resultant readily detectable evidence of growth.

A review of the reports of outbreaks of food infection occurring abroad and in the United States has revealed relatively few which make any mention of the physical condition of the food and whether the consumer had any warning that the contaminated food responsible for the outbreak was unfit for consumption. However, the reports which have included this point practically all agree that the food appeared normal at the time of consumption and that there was nothing to excite suspicion as to its quality. Gaertner,[7] in his original description of B. enteritidis and the classic "meat poisoning" outbreak at Frankenhausen, Germany, stated that to all appearances the meat could not be differentiated from normal meat. Kaensche[8] recorded an extensive outbreak at Breslau attributed to chopped beef contaminated with B. enteritidis, and stated that the meat presented no abnormal appearance either in color, odor, or consistency. A few years later Bowes and Ashton,[9] in the report of an outbreak caused by veal pies contaminated with B. enteritidis, mention that "practically no one complained of the taste or appearance of the pies, which had no smell whatever." Corned beef (Barker[10]) and soup made from meat (Curschmann[11]) contaminated with B. enteritidis were held responsible for several outbreaks where the evidence given by the persons affected pointed to the conclusion that the meat was apparently normal. Williams,[12] recording an outbreak caused by the consumption of pork pies contaminated with B. paratyphosus B[13] (B. aertrycke?), states that "no person complained of the pies at the time of eating;

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[7] Quoted by Müller, M.: Ztschr. f. Hyg. u. Infektionskr., 1910, 8, p. 251.

[8] Ztschr. f. Hyg. u. Infektionskr., 1896, 22, p. 53.

[9] Brit. Med. Jour., 1898, 2, p. 1456.

[10] Ibid., 1899, 2, p. 1367.

[11] Ztschr. f. Hyg. u. Infektionskr., 1906, 55, p. 295.

[12] Jour. Royal Inst. Public Health, 1910, 18, p. 725; see also Trammsdorff, Rajchmann, and Porter, Jour. Hyg., 1911, 11, p. 89.

[13] In many of the earlier outbreaks the causative organism, if positive agglutination tests were secured with paratyphoid B serum, was not differentiated by absorption tests from the true human B. paratyphosus B, or B. schottmülleri.

indeed, the evidence went to show that they were apparently good and that there was nothing unusual in the taste." McWeeney[14] makes a similar statement in his report of an outbreak traced to B. enteritidis beef and also records some of his own experiments in which he found that the strain of B. enteritidis obtained from the outbreak multiplied and spread rapidly in the beef while the meat exhibited no perceptible alteration. An extensive outbreak traced to the consumption of sausage from which B. paratyphosus B[13] was obtained is reported by Federschmidt.[15] The evidence collected from those affected indicated clearly that there was nothing suspicious about the taste or smell of the sausage.

The reports of outbreaks in which foods other than meat were responsible for conveying the infective organism show that the same conclusion may hold true in regard to various miscellaneous foodstuffs. An apple pudding (containing also milk, eggs, and other ingredients) contaminated with an organism quite similar to B. paratyphosus B was said (Vagedes[16]) to present no evidence of contamination. A sour taste noted by some of the affected persons was believed to be due to the apples. One of the most extensive outbreaks occurring in this country was traced to contaminated pie dough by Bernstein and Fish[17] who report that there was "no unusual taste or odor to the pies to excite suspicion." A recent outbreak in France followed the use of cream containing B. paratyphosus B[13] as an ingredient in preparing cakes (Lesné, Violle, and Langle[18]). Here again the evidence is similar to that previously reported. Sewell, Smith and Priestley[19] give an account of an outbreak in an English army hospital attributed to the contamination of unsweetened canned milk with B. aertrycke, mutton strain. They state that cultures of this organism isolated from feces, vomitus, and postmortem were found to be able "to grow freely in the particular brand of milk used, at room temperature, and without changing the appearance or smell of the milk."

On the other hand, there are several observations in the literature which are contradictory, at least in part, to the foregoing statements. Kerr and Hutchens[20] while studying an outbreak traced to B. enteritidis in raw market milk, found that a number of the affected persons volunteered the information that the particular lot of milk under suspicion had had a peculiar taste. Rosenau and Weiss[21] quite recently have reported an outbreak following the consumption of bread pudding from which B. enteritidis was isolated. It is interesting to note that 4 of the 18 persons affected said that "the bread pudding had a peculiar taste" and one of them refused to eat it after the first spoonful. Since there is no evidence in either of the cases mentioned in the foregoing that the peculiarity of taste was due especially to the presence of B. enteritidis, rather than to the development of any one member of an undoubtedly large miscellaneous flora, these two instances, while exceedingly interesting, do not seriously detract from the general conclusion reached on the review of the cases cited.

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[14] Brit. Med. Jour., 1909, No. 2524, p. 1171.

[15] München. med. Wchnschr., 1920, 67, p. 814.

[16] Klin. Jahrb., 1905, 14, p. 517.

[17] Jour. Am. Med. Assn., 1916, 66, p. 167.

[18] Presse méd., 1920, No. 74, p. 725.

[19] Jour. Roy. Army Med. Corps, 1920, 34, p. 510.

[20] Proc. Royal Soc. Med., 114 (Epidem. Section), 7, p. 171.

In addition to the foregoing observations made in connection with "food poisoning" outbreaks, several additional statements appearing in the literature should be included here. In 1898, Dunham[22] in a discussion of "meat poisoning," stated that "a mere naked eye inspection of the meat will not exclude danger, since meat infected with B. enteritidis may have quite a good appearance." Cathcart,[23] discussing the toxin of the Gaertner bacillus, remarks that food contaminated with this organism is "quite without smell." In the course of a study of the souring of beef, Bunyea[24] inoculated pieces of sterile normal beef with several members of the paratyphoid group. After 4 days' incubation at 37 C. no perceptible odor was produced.

These various reports, then, corroborate the conclusion reached as a result of the present work, and it appears that extensive multiplication of the paratyphoid-enteritidis group of organisms may take place in most foodstuffs with little or no alteration in either appearance, odor, or, perhaps, taste. As a result there will be little or no gross physical evidence to warn either the person preparing the meal or the consumer of possible danger.

This is decidedly in contrast to our opinion of most foodstuffs contaminated with botulinus. Many of the recent reports of outbreaks of botulism state that the infected food was noted to be abnormal with respect to either the odor, appearance, or swelled condition of the container. Experimental inoculation of several foodstuffs in the laboratory has verified this. Since most of the botulinus foodstuffs were preserved either in tins or glass containers, it is evident that there was an accumulation of metabolic products of growth, as for instance odors or gases which serve to mark the food as "spoiled." In contrast to this the foods —meat, milk, puddings, pastry, soups, etc.—which commonly have been contaminated with one or another member of the Gaertner group, are not canned. Thus, aside from fundamental differences in the metabolism of the two groups of "food poisoning" organisms, the conditions under which the food is preserved or held no doubt play an important rôle in determining the extent of gross physical evidence of spoilage, one of the important links in the chain of protection afforded the consumer. Having seen that Gaertner group organisms usually give no evidence of their presence in a foodstuff, it does not seem amiss to emphasize the necessity for thorough cooking of all foods, especially those which have been exposed to an incubation period. The instances are numerous in which these organisms, after gaining entrance to a food, have survived a period of cooking insufficient to cause the penetration of heat to the center of the mass.

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[21] Jour. Am. Med. Assn., 1921, 77, p. 1984.
[22] Brit. Med. Jour., 1898, p. 1797.
[23] Jour. Hyg., 1906, 6, p. 112.
[24] Jour. Agri. Res., 1921, 21, p. 689.

SUMMARY

The aim of the present investigation has been to gain some idea of the ability of several type strains of the paratyphoid-enteritidis group to develop in miscellaneous foodstuffs, such as various vegetables, fruits, meats, and evaporated milk. The effects of different conditions, such as temperature of incubation, the hydrogen-ion concentration and the texture of the food, were considered in relation to multiplication and the ability to spread throughout the foodstuff.

All the strains of the Gaertner group multiplied readily in the liquor of several common cooked vegetables, with the exception of the highly acid sauerkraut. In the fruit juices a rapid destruction of the organisms occurred. In several meat products Gaertner group organisms exhibited a marked ability to spread from one original point of inoculation throughout the foodstuff, although this occurred only under optimum temperature conditions.

The development of the Gaertner group in foodstuffs is usually not accompanied by visible alteration or spoilage. The present observations on this point are in accord with the reports of previous outbreaks of "food poisoning" caused by this group of organisms.